IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:		§		
		§		
	Luba COHEN	§		
		§		
Serial No.:	09/955,933	§		
		§		
Filed:	September 20, 2001	§	Group Art Unit:	1651
		§		
For:	LICORICE EXTRACT FOR USE AS A MEDICAMENT	§		
		§	Attorney Docket:	37229
Examiner:	Deborah K. Ware	§		

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF PROF VAYA UNDER 37 CFR 1.132

1. I, Prof. Jacob Vaya am a senior researcher at Migal Galilee Technology Center since 1985. I have been investigating the chemical components of different plant extracts for the last 22 years. My CV is attached to this declaration.

2. Before giving this declaration I carefully read the article by Fuhrman et al., published at Am. J. Clin. Nutr. 1997; 66:267-75, of which I am a co-author, (hereinafter Fuhrman) and U.S. patent No. 6,280,776 to Sha et al. (hereinafter "Sha").

3. Fuhrman describes lowering the susceptibility of Low density lipoprotein (LDL) to oxidation by administering a water-insoluble licorice extract. LDL is the major cholesterol carrier in the blood.

4. Fuhrman describes that lowering LDL susceptibility to oxidation occurs similarly by administrating glabridin, the major active constituent of the licorice water insoluble extract (Fuhrman extract).

5. Fuhrman describes that supplementation of either water insoluble licorice extract or its major active constituent glabridin, results in reduction in the atherosclerotic lesion area of mice aortic arch.

6. Fuhrman concludes that the above beneficial effect of the water insoluble licorice extract is due to its glabridin content.

7. Glabridin is a practically water insoluble compound and it can be extracted with high efficiency from the pulp which remains after water extract of the root. (Starting with water extraction of the root which removes all the water soluble components, and subsequently performing a second extraction using organic solvent which extracts the non-polar constituents remain in the pulp containing mainly glabridin). It is therefore unlikely to find glabridin in a water extract as that used by Sha.

8. Sha describes improving blood cholesterol level, blood sugar, and liver functions as achievable by taking a food supplement that contains up to 15% water-soluble licorice extract.

9. I refer herein to the licorice extract used by Sha as Sha's licorice extract, and to the conditions that Sha describes as being treated by a food supplement that contains Sha's extract – as Sha's conditions.

10. I refer herein to the licorice extract used by Fuhrman as Fuhrman's extract.

11. I was asked if common sense could lead me to replace Sha's extract with Fuhrman's extract in order to treat Sha's conditions. My answer is absolutely not. In the following I explain why this is so.

12. First of all, Sha explains that the main active ingredient in licorice is glyzyrrhizinic acid. Fuhrman describes her extract to be free of glyzyrrhizinic acid. It is against common sense in my field to take constituent X of a successful composition, and replace it with a constituent Y, that is free of the main active ingredient in X. There is actually no sense in doing so.

13. In accordance with Fuhrman, the main active agent in the Fuhrman extract is glabridin. Fuhrman describes that in *in vitro* and *in vivo* experiments, glabridin alone performed similar beneficial effects as did Fuhrman extract. In experiment I carried out with water-soluble extract of licorice, which I believe to be similar to that obtained by Sha, I found that glabridin is practically absent from the water-soluble licorice extract.

14. In summary, laboratory tests I have run showed that the constituent which is reported by Sha to be the active ingredient in Sha's extract is practically absent from Fuhrman's extract, and the component reported by Fuhrman to be the active ingredient in Fuhrman's extract is practically absent from Sha's extract.

15. Additionally, Fuhrman's extract and Sha's extract are different not only in content of glyzyrrhizinic acid, but in nearly all their constituents. Therefore, there is no reason to believe that the effect of one of them will be similar to the effect of the other, even if glyzyrrhizinic acid is irrelevant to the efficacy of Sha's extract.

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16. It is clear to me that the two extracts are very different chemically based on the differences in their water solubility. In detail:

a. Sha's extract is water soluble. This means that most of it constituents are water-soluble substances.

b. Fuhrman's extract is water insoluble. This means that it has very little, if any, water-soluble substances.

c. From a. and b. above, it is straightforward that the two extracts are very different in their chemical constituents.

17. I was asked would one of ordinary skill in my field be motivated to replace Sha's water soluble extract with Fuhrman's water insoluble extract in order to improve shelf-life of the product. My answer is absolutely not, for the reasons detailed below:

a. From my experience I know that shelf-life of plant extracts is not correlated with their water solubility. Therefore, one of ordinary skill in my field would not expect lengthening the shelf-life of a product by replacing a water soluble ingredient with a water insoluble one, unless there is specific information suggesting that the replacement at issue would indeed lengthen the shelf-life. I don't know of the existence of such specific information.

b. I never heard on shelf-life problems associated with water soluble licorice extracts.

c. Even if one component has a longer shelf-life than another, a person of ordinary skill would not suggest exchanging between the two if they have as vastly different chemical constitution as the extracts of Sha and Fuhrman have.

18. Finally, I would like to comment that Sha's extract is very well known in my field, and has been used in folklore medicine for centuries. It is commercially available and being important component in the tobacco industry, and may be marketed without posting any unexpected regulatory problems. Fuhrman's extract, on

the other hand, is not so well known, and the regulatory authorities may be expected to post heavier requirements before allowing its marketing. Therefore, I believe that market forces join the other factors I explained above in challenging the desirability of the suggested replacement.

19. In conclusion, I believe that the idea of replacing Sha's extract with Fuhrman's extract is against common sense.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United states Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Vaya Jacob

2 January, 2008
Date

Prof. Jacob Vaya

Encl.:
Curriculum Vitae

CURRICULUM VITAE

1. Personal Details:

Date of birth:	October 1946
Country of birth:	Iraq
Identity No.:	74158056
Nationality:	Israeli
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2. Higher Education

1968-1971: BSc, Hebrew University of Jerusalem, Faculty of Chemistry, study in Chemistry and Biochemistry.

1971-1973: MSc, Hebrew University of Jerusalem, Faculty of Chemistry, Supervisor: Prof. Albert Zilkha; Thesis: "Sulfonation of Polyamides".

1974-1978: PhD, Weizman Institute of Science, Rehovot, Faculty of Chemistry, Supervisor: Prof. Mario D. Bachi; Thesis: "Investigations on β-Lactams Structurally Related to Penicillins and Cephalosporins". Major accomplishment - Total synthesis of (±)spirocyclopentanodinorpenicillin, synthesis of novel types of compounds: the thiomalonimides.

3. Additional Education, Training and professional certificates

1981-1985: Group Manager; **Teva** Pharmaceutical Industries. Research activities: isolation, purification and structure determination of new compounds isolated from bacteria. Project manager in development and improvement of processes for the production of different types of drugs (**attached list of internal publications**). Optimization of parameters, active involvement in pilot plan for production of drugs.

1985-1990: Team-Head; Migal - Galilee Technological Center.

Research activities: Development of processes and facilities for the production of essential oils and oleoresins from natural sources, optimization of processes from laboratory scale to pilot and eventually commercialization. Development of analytical methods for the identification and quantification of compounds from natural raw materials. Major accomplishment - Setting up commercial enterprise (owned by Israel Chemicals, Ltd.) for production of extract from natural raw materials (roots, seeds, fruits, plants, algae etc.). Our group was responsible for all the R&D and the QC activities to the enterprise.

Processes developed under the above activities:

a. Establishment of efficient installation for the production of essential oils in several tons of raw materials for batch.

b. Extraction and isolation of pigments (carotenoids) from paprika fruit as industrial process (achieving $1x10^6$ c.u.).

c. Development of process for the isolation of β-carotene from dry or paste algae (more than 80% β-carotene in the extract).

d. Development of efficient process for the selective removal of pesticides from ginseng extract (an industrial process).

e. Development efficient processes for the isolation of Partenolide from feverfew, eleutherosides B and E from eleutherococcus, flavonoides from licorice.

f. Development formulations from oleoresins for specific use (solubility, synergism, etc.).

g. Identification and testing of natural antioxidants for therapeutic uses. The effects of natural antioxidants on LDL (the main carrier of cholesterol in human plasma). Development of methods for early identification of atherosclerosis and markers for the study of oxidative stress. Correlation between oxysterols (oxidized cholesterol derivatives) and atherosclerosis development. Studying for correlation between structure of flavonoids and their ability to prevent LDL from oxidation (SAR). Studies on new natural

compounds, their isolation, structure elucidation and their *in vitro* and *in vivo* estrogen-like activity (phytoestrogens). The relation between the structures of phytoestrogens isolated from plants to their activities as agonist/antagonist to estradiol using computerized modeling (Insight, CeriousII). Developing markers for early diagnosis of oxidation damage in humans.

h. Isolation of natural whitening agents. The design and synthesis of new tyrosinase inhibitors.

i. Designing and synthesis of exogenous markers for the characterization of oxidative stress.

4. Appointments at academic institutions

1978-1981: Research Associate with Prof. T. Ross Kelly, Department of Chemistry, Boston College, Boston, Mass. USA. Field of research: Total synthesis of natural compounds. Major accomplishment - An efficient regiospecific total synthesis of Daunomycinone.

1998 (June-Oct). Partial sabbatical, Dept. Molecular Cell Biology at UC Berkeley. Studying interactions between flavonoids and proteins. Developed analytical methods for quantification of Pycnogenol. Established cooperation between our group at Migal and Prof. L. Packer group at Berkeley.

2001- Partial Sabbatical – Research Associate with Profs. A. Sevanian and E. Cadenas. School of Pharmacy, University of Southern California. The effect of phytoestrogens, in vivo models.

2005- Partial Sabbatical – , Centre for Neurotranslational Research, McGill University and LDI for Medical Research, Jewish General Hospital – Prof. Hyman Schipper. Establishing collaboration between the two groups investigating HO-1 and Alzheimer disease.

2003 – Received - Associate Professor

5. Administrative positions in academic institutions

2002-2005 – Head of the Dept. Biotechnology and Environmental Science, School of Science and Technology, Tel Hai Academic College.

6. Positions in non academic & research Institutions

1981-1985: Group Manager; Teva Pharmaceutical Industries (Israel).

1985-1990: R&D manager; Migal - Galilee Technological Center. Establishing factory producing flavors and fragrances – Galilee Aroma LTD.

7. Membership in professional organizations

1990- The Israeli Society of Oxygen and Free Radicals research

1995- The International Society of Free Radicals Biology and Medicine

1996- The European Atherosclerosis Society

1997- The Israeli Atherosclerosis Society

8. Research Grants

Date	Funding agency	Research topics	Grant - US $
1998-1999	Ministry of industry and commerce, Chief science	Phytoestrogens	240,000
2000-2001	Ministry of industry and commerce, Chief science	SAR in phytoestrogens	55,000
1998-2001	Ministry of Science-	Construction of bank of natural extracts.	75,000
2000-2003	Israeli Science Foundation	Agonist and antagonist to estradiol. SAR	135,000
2001-2002	Ministry of industry and commerce, Chief science	New Whitening agents	100,000
20001-2003	Ministry of Science Culture & Sport division for Agricultural and Environment	Development of inhibitors for tyrosinase	80,000
2004-2007	Israeli Science Foundation	The search for natural substrates of Paraoxonase	65,000
2004-2005	D-Cure	The effect of PON1 on early advances glycation products	54,000
2006	Ministry of industry and commerce, Chief science	Masking of protein surfaces for decreased antigenicity	130,000
2007	Ministry of industry and commerce, Chief science	Masking of protein surfaces for Increasing antigenicity	130,000

9. Teaching at Academic institutions

1990-1991: Tel Hai College, Upper Galilee, Israel. Course: Essential oils and extracts in the flavors and fragrances industry.

1992-1993: Tel Hai College, Upper Galilee, Israel. Courses: Antioxidants, chemistry, production and uses in the food and cosmetic industry.

1993-2005: Department of Biotechnology and Environmental Science, School of Science and Technology (B.Sc.), Tel Hai Academic College, Upper Galilee, Israel. Course: Organic Chemistry.

2003-2005: Department of Biotechnology and Environmental Science, School of Science and Technology (B.Sc.), Tel Hai Academic College, Upper Galilee, Israel. Course: Free Radicals and Oxidative Stress.

10. Supervision of Graduate Students

1990-1992. Aviv Cohen, "Preparation of Black pepper emulsion", M.Sc., Hebrew University of Jerusalem, School for Science and Technology, Cassali Institute for Applied Chemistry, with Prof. N.Garti and A. Aserin; Completed.

1991-1993. Vered Dangur, "Palladium Catalysis Elimination reactions", M.Sc. Faculty of Chemistry, Technion, Haifa, with Prof. E.Keinan; Completed.

1995-1998. Paula Belinky, "Effect of antioxidant constituents from the roots of *Glycyrrhiza glabra* (Licorice) on the oxidation of low density lipoprotein (LDL)", Ph.D. School of Medicine, Technion, Haifa, with Prof. M.Aviram; Completed.

1997-1999. Ayelet Nir, " Influence of stress on antioxidants defense system in apple; extraction and separation of components, and examination of their control of superficial scald". M Sc. Hebrew University of Jerusalem, Faculty of Pharmacy, with Dr. Roni Cohen, Prof. Ruth Ben Arye and Amos Levin. Completed.

2001-2006. Ohad Nerya, "Prevention of browning in plant tissues with new natural and synthetic inhibitors of tyrosinase". Ph.D. student, Hebrew University of Jerusalem, Dept. of Fruit Storage Research, with Prof. Ruth Ben Arye. Completed

2001-2005. Andrea Shochtman, " Characterization of oxidative stress processes and their products by chemical synthesized markers". Ph.D. student. School of Medicine, Technion, Haifa, with Prof. Michael Aviram. Completed

2006- Yuval Aluf - MSc. student. School of Medicine, Technion, Haifa, with Prof. John Finberg.

2006- Hagi Tabory- Ph.D. student. School of Medicine, Technion, Haifa, with Prof. Michael Aviram.

11. List of Publications:

11.1. Master and Doctoral Dissertation

M.Sc. – Sulfonation of polyamides. The Hebrew University, Jerusalem. Supervisor: Prof. Albert Zilkha.

Ph.D. Investigations on β-Lactams Structurally Related to Penicillins and Cephalosporins. The Weizman Institute of Science, Rehovot, Israel. Supervisor: Prof. Mario D. Bachi;

11.2. Books (Academic)

1. **Vaya J.** Packer L. 999. Entry on "Antioxidants" in McGraw-Hill Encyclopedia of Science & Technology, 9th Ed, Volume 2. Appearing in 5 languages, as a multi-media CD-ROM and in an on-line version.

2. Jacob Vaya, Snait Tamir, Dalia Somjen, Estrogen-Like Activity of Licorice Root Extract and its Constituents . Oxidative Stress and Disease **(2004),** 14 (Herbal and Traditional Medicine), 615-634. Editors; Lester Packer, Choon Nam Ong and Barry Halliwell, Publisher- Marcel Dekker Inc.

3. Michael Aviram. **Jacob Vaya** and Bianca Fuhrman. Licorice root flavonoid antioxidants reduce LDL oxidation and attenuate cardiovascular diseases. Oxidative Stress and Disease **(2004),** 14(Herbal and Traditional Medicine), 595-614. Editors; Lester Packer, Choon Nam Ong and Barry Halliwell, Publisher- Marcel Dekker Inc.

4. N.P. Seeram, Y. Zhang, Jess D. Reed, Christian G. Krueger, Erika Salas and **Jacob Vaya.** Phytochemical Constituents of Pomegranate (*Punica granatum* L.). **2006.**

5. Jacob Vaya. Novel designed probes for the characterization of oxidative stress in biological fluids, cells and tissues. Advanced Protocols in Oxidative Stress **(2008),** Editor; Donald Armstrong. Humana press, Totowa , New Jersey.

11.3 Articles in refereed journals

1. **Vaya J.,** Zilkha A. 1974. Sulfonation of polyamides. *Isr. J. Chem*. **12**: 873-878

2. Bachi M.D., **Vaya J.** 1976. Azetidin-2-oxo-4-thiones: Novel thermolytic product of β-lactam sulfoxides. *J. Am. Chem. Soc*. 98: 7825-7826

3. Bachi M.D., **Vaya J.** 1977. Reactions and properties of azetidin-2-oxo -4-thiones. *Tetrahedron Letters*, 2209-2212.

4. Bachi M.D., Frydman N., Sasson S., Stern C., **Vaya J.** 1997. Synthesis of (±)-penicillin and (±)-2-spirocyclopentanobisnorpenicillin systems. *Tetrahedron Letters*, 641-644.

5. Bachi M.D., **Vaya J**. 1979. Phosphinimines as useful intermediates in the synthesis of 3-(acylamino)-β-lactams. *J. Org. Chem*. **44**: 4393-4396.

6. Bachi M.D., Goldberg O., Gross A., **Vaya J.** 1980. Synthesis of 4-thioxo-2-azetidinones. *J. Org. Chem*. **45**: 1477-1485.

7. Bachi M.D., Goldberg O., Gross A., **Vaya J**. 1980. Properties and reactions of 4-thioxo-2-azetidinones. *J. Org. Chem*. **45**: 1481-.

8. bachi M.D., Sasson S., **Vaya J.** 1980. Studies related to penicillins and cephalosporins, part 6. Synthesis of the (±)dinorpenicillin-spirocyclopentane system. *J. Chem. Soc. Perkin I*: 2228-2232.

9. Kelly T.R., **Vaya J.,** Ananthasubramanian L. 1980. An efficient, regiospecific synthesis of (±)-daunomycinone. *J. Am. Chem. Soc*. **102:** 5983-5984.

10. Kelly T.R., Behforouz M., Echavarren A., **Vaya J.** 1983. Synthesis of the Rifamycin chromophore. *Tetrahedron Letter*, 2331-2334.

11. Kelly T.R., Ananthasubramanian L., Borah K., Gillard W.J., Goerner N.R., King P.F., Lyding M.J., Tsang W.G., **Vaya J.** An efficient regiospecific synthesis of (±)-daunomycinone. *Tetrahedron.* 40: 4569-4577.

12. Keinan E., Kumar S., Dangur V., **Vaya J**. 1994. Evidence for a cyclic mechanism in (η^3-allyl) palladium chemistry. Promotion of β-hydride elimination by unsaturated organometallics. *J. Am. Chem. Soc*. **116**: 11151.

13. Masaphy S., Levanon D., **Vaya J**., Henis Y. 1993. Isolation and characterization of a novel Atrazine metabolite produced by the fungus Pleurotus Pulmonarius, -chloro-4-ethylamino-6-(1-hydroxyisopropyl)amino-1,3,5-triazine. *Appl. Environ. Microbiol*. 59: 4342-4346.

14. Degani G., Gal E., **Vaya J.** 1994. In vitro biosynthesis of steroids in ovary of asymchronic Trichogaster trichopterus (Pallus 1770). *Comp. Biochem. Physiol.* 109B: 715-723.

15. Vaya J., Belinky P., Aviram M. 1997. Antioxidant constituents from licorice roots: isolation, structure elucidation and antioxidative capacity toward LDL oxidation. *Free Radic. Biol. Med.* 23: 302-313.

16. Fuhrman B., Buch S., **Vaya J.**, Belinky P., Coleman R., Hayek T., Aviram M. 1997. Licorice alcoholic extract and its major polyphenol glabridin protect LDL against lipid peroxidation: in vitro and ex-vivo studies in human and in the atherosclerotic apolipoprotein E deficient mice. *Amer. J. Clin. Nutr.* 66, 267-275.

17. Hayek, T., Fuhrman, B., **Vaya, J.**, Rosenblat, M., Belinky, P. A., Coleman, R., Elis, A. and Aviram, M., 1997. Reduced progression of atherosclerosis in the apolipoprotein E deficient mice following consumption of red wine, or its polyphenols quercetin or catechin, is associated with reduced susceptibility of LDL to oxidation and to aggregation, *Arterioscler. Thromb. Vasc. Biol.* 17, 2744-2752.

18. Belinky P., Aviram M., **Vaya J.** 1998. The antioxidative effects of the isolated isoflavan Glabridin on endogenous constituents of LDL during its oxidation. *Atherosclerosis.* 137, 49-61.

19. Belinky P., Aviram M., Saeed Mahmood., **Vaya J.** 1998. Structural aspects of the inhibitory effect of glabridin on LDL oxidation. *Free Radic. Biol. Med.* 24, 1419-1429.

20. Rosenblat, M.; Belinky, P. A.; **Vaya, J.**; Levy, R.; Merchav, S.; Aviram, M. Macrophage enrichment with the isoflavan glabridin inhibits NADPH oxidase-induced cell-mediated oxidation of low density lipoprotein. A possible role for protein kinase C. *J. Biol. Chem. 1999 May 14;274(20):13790-9*

21. Hadi Moini, Antonio Arroyo, **Vaya, J.**; Lester Packer. 1999. Bioflavonoid effects on the mitochondrial respiratory electron transport chain and cytochrome c redox state. *Redox Report.* 4, *35-41.*

22. Fuhrman Bianca., **Vaya J.**, Belinky Paula., Aviram Michael. **1999**. The isoflavan glabridin inhibits LDL oxidation : structure and mechnistic aspects. *Spec. Publ.-R. Soc. Chem.* 240 (Natural antioxidants and anticarcinogens in nutrition, health and disease), 161-165.

23. Irit Maor., Marielle Kaplan, Tony Hayek., **Vaya. J.**, Aaron Hoffman and Michael Avirm. Oxidized monocyte-derived macrophages in aortic atherosclerotic lesion from apolipoprotein E-deficient mice and from human carotid artery contain lipid Peroxides and Oxysterols. *Biochem Biophys Res Commun* **2000**;269(3):775-780

24. Michael Aviram, Emiliya Hardak, **Vaya, J.**, Saeed Mahmood, Simcha Milo, Aaron Hoffman, Scott Billicke, Dragomir Draganov and Mira Rosenblat. Human serum paraoxonases (PNO1), Q and R selectively decrease lipid peroxides in human coronary and carotid atherosclerotic lesions: PON1 esterase and peroxidase-like activities. *Circulation.* **2000** May 30;101:2510-2517.

25. Snait Tamir, Mark Eizenberg, Dalia Somjen., Naftali Stern, Rayah Shelach, Alvin Kaye, and **Vaya, J.**, Estrogenic and Antiproliferative Properties of Glabridin from Licorice in Human Breast Cancer Cells. *Cancer Research*. **2000**, 60, 5704-5709.

26. Vaya, J., Saeed Mahmood, Tony Hayek, Ehud Grenadir, Simcha Milo, Aaron Hoffman, and Michael Aviram. Selective distribution of oxysterols in human plasma, plasma lipoproteins and atherosclerotic lesions. *Free Radical Research.* **2001**; 34, 485-497.

27. Kaye AM, Spatz M, Waisman A, Sasson S, Tamir S, **Vaya J.**, Somjen D. Paradoxical interactions among estrogen receptors, estrogens and SERMS: mutual annihilation and synergy. *J Steroid Biochem Mol Biol.* **2001**; 76(1-5):85-93.

28. Aviram Michael and **Vaya J.,** Markers for low-density lipoprotein oxidation. *Methods Enzymol.* **2001**;335:244-256.

29. Snait Tamir, Mark Eizenberg, Dalia Somjen, Sarit Izrael and **Vaya, J.,** Estrogen Like-Activity of Glabrene and other Constituents Isolated from Licorice Root. *J Steroid Biochem Mol Biol.* **2001.** 78:291-298.

30. Vaya, J., and Michael Aviram. Nutritional Antioxidants: Mechanisms of Action and Medical Applications. *Current Medicinal Chemistry-Immunology, Endocrinology & Metabolic Agents.* **2001.** 1:99-117

31. Kaplan M, Hayek T, Raz A, Coleman R, Dornfeld L, **Vaya J.**, Aviram M. Pomegranate juice supplementation to atherosclerotic mice reduces macrophage lipid peroxidation, cellular cholesterol accumulation and development of atherosclerosis. *J. Nutr.* **2001.** 131:2082-2089.

32. Snait Tamir, Sarit Izrael, **Vaya, J.**, The Effect of Oxidative Stress on ERα and ERβ Expression. *The Journal of Steroid Biochemistry and Molecular Biology* . September **2002**. 81: No 4-5, ;327-332.

33. Vaya, J., Somjen, D.; Tamir, S. The role of the isoflavan's 2' hydroxyl in diverse biological activities. Editor(s): Pasquier, Catherine. Proceedings of [the] Biennial Meeting of the Society for Free Radical Research International, 11th, Paris, France, July 16-20, 2002 (2002), 567-574. Publisher: Monduzzi Editore, Bologna, Italy

34. Vaya, J., Saeed Mahmood., Amiram Goldblum., Michael Aviram, Nina Volkova, Amin Shaalan., Ramadan Musa, Snait Tamir. Inhibition of LDL oxidation by flavonoids in relation to their structure and calculated enthalpy. *Phytochemistry*. **2003.** 62 (1): 89-99.

35. Ohad Nerya, **Vaya, J.,** Ramadan Musa, Sarit Izrael, Ruth Ben-Arie and Snait Tamir.. Glabrene and Isoliquiritigenin as Tyrosinase Inhibitors. *Journal of Agricultural and Food Chemistry*. 51(5): 1201-1207, **(2003)**.

36. Rivka Ofir, Snait Tamir, Soliman Khatib, **Vaya, J.,** Inhibition of serotonin re-uptake by licorice constituents. *The Journal of Molecular Neuroscience*, **(2003)**; 20:35-140.

37. Andrea Szuchman, Michael Aviram, Snait Tamir, **Vaya, J.**, The effects of oxidative stress on cholesterol, linoleic acid or tyrosine vs. their mixture. **(2003).** *Free Radical Research*. 37:1277-88.

38. Vaya, J., and Snait Tamir. The relation between the chemical structure of phytoestrogens and their estrogen-like activities **(2004)**. *Current Medicinal Chemistry*. 11:1333-1343.

39. Ohad Nerya, Ramadan Musa, Soliman Khatib, Snait Tamir, **Vaya, J.,** Chalcones as potent tyrosinase inhibitors. The effect of hydroxyl- positions and numbers **(2004)**. *Phytochemistry*, 65(10):1389-1395.

40. Somjen Dalia, Knoll Esther, **Vaya, J.,** Stern Naftali, Tamir Snait. Estrogen-Like Activity of Licorice Root Constituents Glabridin and Glabrene in Vascular Tissues *In Vitro* and *In Vivo*. **(2004).** *The Journal of Steroid. Biochemistry and Molecular Biology*. 91(3):147-155.

41. Dalia Somjen, Sara Katzburg, **Vaya, J.**, Alvin M. Kaye, D. Hendel, G.H. Posner, Snait Tamir. Estrogenic activity of glabridin and glabrene from

licorice roots on human osteoblasts and prepubertal rat skeletal tissues. **(2004).** *The Journal of Steroid Biochemistry and Molecular Biology*. 91 (4-5), 241-246.

42. Tzchori, Itai; Degani, Gad; Elisha, Ronit; Eliyahu, Rivka; Hurvitz, Avshalom; **Vaya, J.,**; Moav, Boaz. The influence of phytoestrogens and oestradiol-17β on growth and sex determination in the European eel (Anguilla anguilla). *Aquaculture Research*. **(2004),** 35(13), 1213-1219.

43. Soliman Khatib, Ohad Nerya, Ramadan Musa, Snait Tamir, **Vaya, J.**, Chalcones as potent tyrosinase inhibitors. The importance of a 2,4-substituted resorcinol moiety. *Biorganic & Medicinal Chemistry.* **(2005)**. 13(2), 433-441.

44. Rosenblat M, **Vaya, J.,** Shih D, Aviram M. Paraoxonase 1 (PON1) enhances HDL-mediated macrophage cholesterol efflux via the ABCA1 transporter in association with increased HDL binding to the cells: a possible role for lysophosphatidylcholine. **(2005)**. Atherosclerosis. 2005 Mar;179(1):69-77.

45. Nerya O., Ben-Arie R., Dannai O., Tamir S. and **Vaya, J.** Inhibition of mushroom browning. **(2005)**. Acta Hort. 682:1885-1888.

46. Szuchman A, Aviram M, Soliman K, Tamir S, **Vaya, J.**, Exogenous N-linoleoyl tyrosine marker as a tool for the characterization of cellular oxidative stress in macrophages. **(2006).** *Free Radic Res.* Jan;40(1):41-52.

47. Ohad Nerya, Ruth Ben-Arie, Tal Lussato, Ramadan Musa, Soliman Khatib and **Vaya, J.,** Prevention of *Agaricus bisporus* postharvest browning with tyrosinase inhibitors. **(2006).** *Postharvest Biology and Technology*. 39(3): 272-277,

48. Mira Rosenblat, Leonid Gaidukov, Olga Khersonsky, **Vaya, J.,** Roni Oren, Dan S. Tawfik, and Michael Aviram. The catalytic histidine dyad Of HDL-associated serum paraoxonase 1 (PON1) is essential for PON1 - mediated inhibition of LDL oxidation and stimulation of macrophage cholesterol efflux. **(2006)**. *J. Biol. Chem,* 281(11), 7657-7665,

49. **Vaya, J.**, Saeed Mahmood. Flavonoid content in leaf extracts of the fig (*Ficus carica* L.), carob (*Ceratonia siliqua* L.) and pistachio (*Pistacia lentiscus* L.). **(2006),** *BioFactors.* 28(3-4), 169-175.

50. Vaya, J., Wei Song, Soliman Khatib, Guoyan Geng, and Hyman M. Schipper. Effects of Heme Oxygenase-1 Expression on Sterol Homeostasis in Rat Astroglia. **(2007).** *Free Radicals Biology & Medicine*. 42, 864-871.

51. Soliman Khatib, Ramadan Musa, **Vaya, J.,** Linoleoyl tyrosine 2'-deoxyguanosyl ester as a novel designed synthetic probe for the characterization of oxidative stress. **(2007).** *Bioorganic Medicinal Chemistry*. 15 (11), 3661-66.

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54. Andrea Szuchman, Michael Aviram, Khatib Soliman, **Vaya, J.,** Characterization of oxidative stress in blood from diabetic versus hypercholesterolemic patients, using a novel synthesized marker. **(2007).** *Biomarkers.* August 30, 1-13.

11.3.2. Articles accepted (in print)

11.3.3. Articles Submitted.

55. Dalal Alkazemi M.Sc., Stan Kubow Ph.D., **Jacob Vaya Ph.D.**, Sara Meltzer, M.D., Grace Egeland Ph.D. Oxysterols: An Important Marker of Oxidative Stress in Obese Adolescents. **(2008).** Submitted.

56. Hagai Tavori, Soliman Khatib, Michael Aviram, **Jacob Vaya**. Characterization of the PON1 active site using modeling simulation, in relation to PON1 lactonase activity. **(2008).** *Bioorganic Medicinal Chemistry*. Submitted.

57. Michal Efrat, Mira Rosenblat, Saeed Mahmood, **Jacob Vaya** and Michael Aviram. Di-Oleoyl Phosphatidylcholine (PC-18:1) Stimulates Paraoxonase 1 (PON1) Enzymatic and Biological Activities: In vitro and in vivo studies. **(2008).** *Atherosclerosis*. Submitted.

11.3.4. **Patents**

1. Anathasubramanian, Lakshminarayan; Kelly, T. Ross; **Vaya, J.,** Synthesis of daunomycinone and derivatives thereof. (Research Corp., USA). U.S. **(1983)**, 6 pp. CODEN: USXXAM US 4415498 A 19831115. Application: US 79-91897 19791107. CAN 100:85509 AN 1984:85509.
2. **Vaya, J.,** Kaspi, Joseph; Ladkani, David; Salemnick, Gad; Schoenberger, Clara; Yellin, Haim; Cherkez, Stephen. (Orvet B. V., Neth.). 1-Bromoethyl ethyl carbonate and its use in the preparation of 1'-ethoxycarbonyloxyethyl esters of penicillins and intermediates, and 1-bromoethyl chloroformate and 1-bromoethyl bromoformate obtained as intermediates. Eur. Pat. Appl. **(1984)**, 43 pp. CODEN: EPXXDW EP 108547 A2 19840516 Designated States R: AT, BE, CH, DE, FR, GB, IT, LI, NL, SE. Application: EP 83-306482 19831025. Priority: IL 82-67177 19821104; IL 83-67637 19830107. CAN 101:130508 AN 1984:530508
3. **Vaya, J.,** Michael Aviram **(1998)**. Migal Galilee Technology Center. Pharmaceutical compositions containing paraoxonase for the treatment of cardiovascular disorders. Patent application 124079. 14/4/98, Israel.

4. **Vaya, J.,** and Snait Tamir. (**1999**). Phytoestrogenic pharmaceutical preparations. MIGAL Galilee Technological Center. Patent application 9865/pph/99. Submitted for PCT – October **2000** (PCT/IL00/00700).
5. Jacob Pitcovsky and **Vaya, J.,** *Method for obtaining modified proteins and viruses with intact native binding site.* Israeli Patent Application No. 166049, in the name of Gavish-Galilee Bio Applications Ltd. 5 January **2005.** PCT WO 2006/070371 A3
6. **Vaya, J.,** The development of exogenous marker for the characterization of oxidative stress. 31. 05. **2005**, GAVISH-012 US PROV.
   Jacob Vaya, Snait Tamir, Title: Exogenous marker for oxidative stress,
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7. Itschak Lamensdorf, Michael Krakovsky, **Vaya, J.,** Snait Tamir, Use of glabridin and derivatives thereof as neuroprotective agents Pharmaseed Ltd. Our Ref.: PHARM-001 US PROV, March **2006.**
8. **Vaya, J.,** and Hyman Schipper. Upregulation of Heme Oxygenase-1 Expression for the Reduction of Cholesterol. GAVISH-023 US PROV. December **2006.**

11.3.5. Publications in Non-Reviewed Journals (Teva - pharmaceutical industries, 1981-1985).

Vaya J., Schonberger K., Ben-Haim J., Cohen Z., VanDen Broucke E. June 1982. Alternative procedures for preparation of ampicillin Dane K-salt. Teva.

Vaya J., Goldenberg V., Stern D., Ben-David D., Salemnik G. August 1982. Preparation of bacampicillin from ampicillin Dane K-salt. Teva.

Vaya J., Zyk N., Barak G. Sept.1982. Isolation, purification, biological properties and structure determination of compounds N_1 and N_2. Teva.

Vaya J., Goldenberg V. Nov. 1982. preparation of cloxacillin sodium monohydrate. Teva.

Vaya J., Cohen Z., Barak G., Dec. 1982. A preliminary procedure for the preparation of the intermediate product in the synthesis of piroxicam and isoxicam. Teva.

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Vaya J., Goldenberg V., Stern D. April 1983. Preliminary experiments for investigating the two diastereomers of bacampicillin. Teva.

Vaya J., Goldenberg V., Stern D. April 1983. Preparation of bacampicillin hydrochloride using bromodiethylcarbonate of different purity range.

Vaya J., Shaviv F., VanDen Brouke E., Schonberger C. Aug. 1983. preparation of pyrilamine maleate, part I. Teva.

Vaya J., Cohen Z., Barak G. Oct. 1983. Preparation of metroprolol intermediate. Teva.

Vaya J., Cohen Z., VanDen Brouke E., Schonberger C. March 1984. Preparation of aminoglutethimide. Teva.

Vaya J., Cohen Z., Goldenberg V. Oct. 1984. Preparation of pyrilamine maleate, part II. Preparation of z-(n,p-anizyl) aminopyridine. Teva.

Vaya J., Barak G., Hirsch D. Nov. 1994. Preparation of pyrilamine maleate, part III. Optimization of the procedure for the preparation of pyrilamine base. Teva.

Vaya J., Barak G., Hirsch D. Nov, 1984. Preparation of pyrilamine maleate, part IV. Preparation of N,N-dimethyl-2-chloroethane from its salt. Teva.

Vaya J., Barak G., Hirsch D., Schonberger G., Cohen Z., Zilberstein H., Tiram A., Saratana D., Shaviv F. Oct. 1984. Optimization of the procedure for the preparation of aminoglutethimide. Teva (pilot plan), Teva.

12. Participation in Scientific Conferences (During the last 6 years)

Year	Subject	Name of conference/year
2000	Poster/ Human serum paraoxonases	Society for research, prevention and treatment of atherosclerosis/Eilat
2000	Poster/Flavonoids as inhibitors of LDL oxidation/ SAR study	Symposium/ Oxidative stress and atherosclerosis/ Oslo-Norway
2000	Poster/Oxysterols as marker for atherosclerosis development	XIIth international symposium on atherosclerosis/ Stockholm, Sweden
2001	Inhibition of LDL oxidation	II International Symposium on Natural antioxidants –Beijing -China
2001	Poster/The affect of oxidative stress on Estrogen receptors Era and Erb	8th Annual Meeting of the Oxygen Society- Durham, North Carolina - USA
2002	International Atherosclerosis Society	Paris

2003	Free radicals	Ionina- Greece
2004	Lecture/Prevention and treatment of Atherosclerosis	Eilat- Israel
2004	Lecture/ Chalcones as potent whitening agents- SAR study.	XXII International Conference on Polyphenols, Helsinki , Finland
2004	Lecture/ Exogenous tyrosine linoleate marker as a tool for characterization of cellular OS.	Jerusalem, Israel.
2005	Lecture -Marker for Oxidative stress	International Analytical Chemistry Conference. Tel Aviv
2005	Lecture- Marker for oxidative stress Lecture- Marker for Oxidative stress	McGill University, Faculty of nutrition, Montreal McGill University, LDI, The Jewish General Hospital
2006	Lecture -Effects of Heme Oxygenase-1 Expression on Sterol Homeostasis in Rat Astroglia	The 22nd Annual Meeting of the Israel Society for Oxygen and Free Radical Research. Tel Aviv

13. Current research

1. Oxidative stress and diseases –

1.1- : Multifunctional substrate for early detection of oxidative stress susceptibility and application to **Parkinson's disease**. This study utilizes an innovative molecule developed by us to characterize oxidative stress fingerprint of biological tissues. The novel marker constructed from multifunctional molecule of linoleic acid/tyrosine/guanosine (LTG) used to study oxidative/nitrative fingerprint for peripheral blood of Parkinsonian patients by comparison to normal controls, and by comparison to preclinical models of oxidative stress. The research program include experiments designed: a) To establish the oxidative stress profile for LTG in vitro using dopaminergic cell lines and a variety of stressors. b) To establish the oxidative stress profile for LTG in vivo using a rat model of increased dopaminergic turnover (unilateral 6-hydroxydopamine nigral lesion). c) To determine the oxidation/nitration of LTG by patient and control plasma, using blood from volunteer controls and patients of both sexes and of different ages. The controls are chosen to balance the patient group in age and sex. d) Results in patients are also related to severity of symptoms as determined by standard neurological criteria.

Collaborators: Prof. John Finberg, Faculty of Medicine, Technion, Haifa.

1.2. Heme oxygenase-1/sterol interaction in **Alzheimer disease-** Excess brain cholesterol can be eliminated via the ABC1A transporter and HDL cholesterol efflux pathways, and by esterification, oxidation to 24-, 25- or 27-hydroxy cholesterol (oxysterols) or conversion to bioactive steroids. Collaborative data from our

laboratories in Canada (H. M. Schipper) and Israel (J. Vaya) indicate that HO-1 over-expression in astroglia profoundly influences cholesterol and oxysterol metabolism in these cells. **Aims**: Building on our initial data set, experiments have been designed to test the following **hypotheses**: **#1-** HO-1 up-regulation in cultured astroglia suppresses cellular cholesterol levels by inhibiting the cholesterol biosynthetic pathway and/or augmenting cholesterol efflux via LXR activation. **#2-** Decreased cholesterol and cholesterol precursor concentrations and increased oxysterol levels correlate with augmented HO-1 protein expression in AD-affected human brain tissue.

Collaborators: Prof. Hyman Schipper- McGill University, Centre for Neurotranslational Research, Jewish General Hospital, Quebec, Canada.

2. **Method for obtaining modified proteins and viruses with intact native binding site.** **The aim.** The present study relates to methods for obtaining modified proteins, e.g. antibodies, and viruses with an intact native binding site and decreased antigenicity. **The principle idea**: There are several techniques for binding compounds to proteins in order to change proteins properties obtaining (a) reduction of protein immunogenicity, (b) change of the protein's surface properties and (c) increase of the plasma half-life when a protein is supplemented as drug. In most of these methods polymers are used for the conjugation of which two major polymers are the polyethylene glycol and Dextran or Dextran derivatives. The major disadvantage of these coating techniques is the fact that they can't distinguish between the different domains of the protein and bind non-specifically to protein, therefore reduce proteins' activity. However, in this research program we **aim** to overcome this obstacle in a simple and elegant procedure in which proteins and viruses antigenicity is reduced without damaging the native active/binding site of the protein.

Collaborators: Prof. Jacob Pitcovski – Migal Galilee Technology Center.

3. Mutual associations between **Paraoxonase-1 (PON1)** and exogenous lactones. Atherosclerosis is associated with increased oxidative. Oxidized low-density lipoprotein (Ox-LDL), as well as oxidized high-density lipoprotein (Ox – HDL), possesses atherogenic properties. Ox-LDL is taken up by macrophages at enhanced rate, whereas Ox-HDL losses its ability to induce cholesterol efflux from

macrophages, leading to accumulation of cholesterol and formation of foam cells, the hallmark of early atherosclerosis. PON1 in the serum is a HDL-associated enzyme which most of the anti-atherogenic properties of the HDL are thought to relate to PON1 activities, though, PON1 substrate is not yet known. **Aims:** To investigate the effects of PON1 on exogenous substrate such as unique lactonic drugs and nutrients, as well as the effect of such lactonic substances on PON1 structure and activity. Upon incubation of PON1 with exogenous lactones analyses is performed of PON1 anti-atherogenicity, alteration in PON1/substrate structure changes in oxido/redox status and related enzymes and PON1 glutathionization.

Collaborators: Prof. Michael Aviram, School of Medicine, Technion, Haifa.

14. Referees

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3. Prof. Lester Packer. Dept. Molecular Cell Biology, Univ. California at Berkeley, Berkeley, CA, 94720. Tel: 510-642-1872. Fax: 510-642-8313.

4. Professor Hyman M. Schipper, MD, PhD, Department of Neurology and Medicine McGill University. Director, Centre for Neurotranslational Research. Lady Davis Institute for Medical Research. Montreal, QC, Canada. Ph: 514-340-8260. FAX:514-340-7502. hyman.schipper@mcgill.ca